MICROMACHINING OF BIOMEDICAL MATERIALS (SHAPE MEMORY ALLOYS) USING ACIDITHIOBACILLUS FERROOXIDANS AND THIOOXIDANS MICROORGANISM

A SYNOPSIS

Submitted by

PRADEEP M

(Reg. No. 9200009002)

in partial fulfilment for the award of the degree of

DOCTOR OF PHILOSOPHY

DEPARTMENT OF MECHANICAL ENGINEERING

SCHOOL OF MECHANICAL AERO AUTO AND CIVIL ENGINEERING

KALASALINGAM ACADEMY OF RESEARCH AND EDUCATION

(DEEMED TO BE UNIVERSITY)

ANAND NAGAR, KRISHNANKOIL – 626 126

TAMIL NADU, INDIA

JULY 2023

1. INTRODUCTION

Shape memory alloys have become well known for their ability to underg large shape changes that traditional metals could never do. One such alloy, NiTi, has been of particular interest due to it's high efficiency (when compared to other SMA's) to convert thermal energy into mechanical energy. Nickel - Titanium (NiTi) alloy is one of the widely used shape memory alloys (SMA) in aeronautical, electrical, and medical applications. Severe reactions to thermodynamic and mechanical parameters and ability to return to their original shape make these alloys distinctive from other alloy regarding machining ability. New developments in the field of material science have led to new engineering metallic materials, composite materials, high tech ceramics and shape memory alloys, having good mechanical properties and thermal characteristics as well as sufficient electrical conductivity.

Shape Memory Alloy (SMA) is an advanced engineering material which exhibits shape memory effects and other properties. The unique properties such as the shape memory effect (SME) and superelasticity (SE) make NiTi an excellent candidate in many functional designs. Superelasticity, also termed as pseudoelasticity, refers to a phenomenon that certain materials possess. Superelastic materials can return to their initial shape when the applied deformational stress is removed, which leads to a subsequent recovery of the deformation strain, thus the material returns to its original shape. The shape memory effect refers to the recovery of plastic strains in an initially deformed in low temperature material. It is now apparent that shape memory and superelasticity are viable solutions for problems in medical device industry. NiTi also possesses a variety of distinctive qualities, including strong corrosion resistance and biocompatibility.

In order to utilize in industry, NiTi alloy must undergo appropriate machining process. The high strength, high wear resistance, pseudo plasticity and outstanding biocompatibility make the machining of Ni-Ti SMA complicated using conventional methods and machining of Ni-Ti SMA requires non-conventional machining processes such as EDM and laser machining. In non conventional machining process, there also some issues, because of its high strength, strain hardening, and increased heat generation. To overcome these issues another method of machining is needed.

There are several issues occurred in conventional and Unconventional machining process while machining NiTi for medical applications. In biomedical applications, machining is of micron level of material removal, fine surface roughness and fine dimensional tolerances and it cannot be achieved by the above machining processes and it is suited by micromachining process. Micro components are crucial parts of the devices used today in electronics, optics, aerospace and other technologies. Besides their high sensitivity and compactness, the manufacturing process involving micro – components requires lower energy consumption and smaller amounts of raw materials than conventional production.

Non traditional machining processes are becoming more popular now a days in industries for microfabrication. Micro machining process are plays vital role in the manufacturing industries to produce micro components using low power consumption with high sensitivity. In micromachining high density of energy is used to remove a very small amount of material which wastes much energy. Micro manufacturing process is inevitable in the house holding appliance like telecommunication, electronics, medicine, and automotives.

Machining process for material removal, additive and shaping mainly fall into three categories. Micro machining can be done by physical, chemical, and biological energy. Technology of using physical and chemical energy is well addressed and commercialised for the production of micro machined components.Although physical and chemical methods of machining are very fast, they have some impacts on dynamics of process in biotic and abiotic systems outside the boundaries of the work space. It has been observed that microorganisms attack structures and induce corrosion. If this microbe –induced corrosion process can be controlled and guided in a specific direction, an energy efficient and cost effective approach can be developed for material processing application.

The use of biological energy in machining is limited. Since last few decades, chemolithotrophs bacteria have been used by many researchers in biological machining for micro fabrication and reported the first fundamental study on possibility of biomachining by micro level of material removal. In Bio machining, micro organism will be used as source of energy to machine the components and it create lot of openings in the manufacturing sector to produce components with

environmental advantages (green manufacturing). Machining with microorganism gives lot of advantage in the machining process, like production of components with lower energy resources, low cost and no heat affected zone. Heat affected zone is the major issue in case of physical and chemical energy micromachining process.

Acidithiobacillus Ferrooxidans and Thiooxidans microorganisms which are chemolithotrophic bacteria is a rod shaped bacillus bacterium that is about 1 μm long and 0.5 μm in diameter and it used to leach and mobilize metals from solid materials possibly involves three principles: (i) redox reactions, (ii) the formation of organic or inorganic acids, and (iii) the excretion of complexing agents. The reactions involved in biomachining is the ability of Acidithiobacillus Ferrooxidans and Thiooxidans is for metal removal and is occurred in two stages namely Direct and Indirect Mechanism.

M/s. SOM Phyto Pharma Pvt.Ltd, Hyderabad and M/s. Hitech Life Sciences Pvt. Ltd, Nellore gave the technical details of Acidithiobacillus Ferrooxidans and Thiooxidans Microorganisms like preparation of culture, importance of growth rate, handling of equipments like shaker, autoclave and Laminar Chamber and handling of microorganisms with its safety procedure.

The application of this proposed technology would target the medical/ bio medical, electronic industries. In addition to that the technological development in this area would be helpful for making micro moulds, micro valves, actuators, fluidic circuits and motors. Precision in critical while manufacturing components for the aerospace industries, any failure leads to immense safety risk. Those components can be machined by bio machining process.

2. LITERATURE REVIEW

This chapter elaborately discusses the earlier works done in the Shape memory alloys, Machining by conventional and unconventional process with some issues , micromachining with biomachining process. And, also these literature reviews are fulfilling the essential information on natural on biomachining process for our present needs. This chapter explained the different research areas related to different materials involved in Shape memory alloys and Biomachining process. This section deeply analyses the research works done by the previous researchers and previous kinds of publications for the following field:

Daneshmand et al [1] improved the SMA as intelligent materials with unique properties that are superior to other alloys**.** With respect to the machining capability, reactions take place for thermodynamic, mechanical, and the ability to revert to their original size and shape. These processes are comparable to the aforementioned machining techniques that investigated MRR and surface roughness. Metallic SMA have several distinctive properties. It is possible to recover from significant deformations by applying heat (Shape memory effect) or by relieving stress (Super elastic effect). SMA has a low yield strength and is easily deformed into the desired shape when it is cold or below its transformation temperature.

Morkopolous et al [2] provided a thorough explanation of the machining protocol, which shapes parts by removing annoying material that is taken away from the workpiece in the form of chips. In some machining operations, evaporation or ablation occurs. Depending on the machinability of materials, restrictions are compulsory when working with difficult-to-work materials like NiTi.

Guo et al [3] analysing the dynamic mechanical behaviour of NiTinol during cutting, and discovered that extraordinary strength and definite heat are to bear responsibility for severe flank wear and tribochemical crater wear. The deformation results in the austenitic white layer during cutting, whereas quenching results in the twinned martensitic white layer.

. Kuppuswamy [4] investigated the medical fraternity of machining SMA, where microengineering features like micro-slots, grooves, and profiles of size 0.5 mm occur. Also using high-speed micromachining, it is possible to produce NiTi alloy products without compromising quality and productivity of the machined surface and textures. When the alloy transitions occur from the B2 phase to the B19 phase, the machining performance of NiTi alloy is characterized by reduced burr size and low cutting forces.

Velmurugan et al [5] state that shape memory alloys are unique materials due to their characteristics and use in the fabrication of microdevices in the medical and aerospace sectors. Shape memory alloys with accurate dimensions, essential properties and required shape are manufactured in manufacturing sectors using conventional and unconventional machining processes.

Hassan et al [6] machining operations can be divided into a variety of categories. One of these categories is turning, which is a cutting process that eliminates material by creating cylindrical and external forms. If the cutting process concentrates on internal turning, it is referred to as boring for internal shapes, as stated by the aforementioned authors. In an effort to comprehend the conventional machining characteristics,

Weinert [7] investigated different cutting speeds, includes in conventional machining of NiTi results in higher energy consumption when compared to unconventional machining, and it also becomes too environmentally unfriendly. Sustainable cutting-edge processes are required to prevent higher energy consumption during machining operations.

Bisaria [8] reviewed the conventional machining process with various operations such as turning, drilling, and milling with variables of cutting speed and feed. Traditional machining has unexceptional properties and also presents several challenges such as surface finish, increased machining time, poor tool failure and strain hardening.

Manjaiah et al [9] reported that conventional machining for NiTi is tough due to the tool wear properties and increased heat exposure to the workpiece, which affects the end quality. To overcome these difficulties in conventional machining, advanced machining process such as electrical discharge machining (EDM) and wire (WEDM), and laser Machining are chosen for processing NiTi SMA. By producing less thermal impact than conventional machining, advanced machining produces excellent outcomes in terms of material removal rate (MRR) and surface roughness (SR).

Theisen [10] investigated at MRR and surface-related modifications when using EDM to machine NiTi. The selection of current and frequency is required to get the maximum MRR with superior surface properties. A high current was used to attain the maximum MRR, and the current value can be increased to increase MRR and to enhance energy for surface roughness, generate deep and wide craters. The wider craters increase SR by enhancing current intensity.

Uno et al [11] who determined that the Thiobacillus Ferroxidans can be used to machine a groove on copper and pure iron materials. The result of first experiments on biological machining reveled that the depth of the grooves increased linearly with

machining time. On the same study, another approach was also used to understand the effectiveness of electric field assisted bio machining process. It is found that, later approach was provided the better metal removal rate than the former approach.

Pallvi Verma et al [12] changed the microorganisms with same aluminium alloy 4004 with Aspergillus niger and the same SMRR was analyzed for same time intervals and SMRR is best at 48hrs of machining process. The parameters such as concentration, shaking rate and temperature cane be varied to get best machining rate.

Miyano et al [13] used natural bacteria with high density obtained from mines drainage water. Mines drainage water contains lot of Thiobacillus Ferrooxidans which include high ferric and sulfur content. The effect of indirect mechanism on material removal rate on low carbon steel is discussed.

Daniel Johnson et al [14] studied the effect of surface roughness and material removal rate while machining the pure copper using Acidthiobascillus Ferrooxidans. They employed novel Most Probable Number (MPN) to count the microorganism and subsequently correlated with the experimental results and they concluded that the material removal rate was very slow and surface roughness of the material deteriorates irrespective of initial surface roughness.

Chang et al [15] first observed the physical contact between the metals and chemolithotropic acidithobacillus Ferrooxidans and Thiooxidans. The dissolution of Cu, Al, and Ni remarkably higher in acidithiobacillus Ferrooxidans and thiooxidans compared to chemical etching.

Istiyanto et al [16] established the relationship between material removal rate and machining time while machining the Cu in the presence of A. Ferrooxidans. It is found that the material removal rate is inversely proportional to machining time, not follows the liner line. The reason could be due to the depletion of oxygen for the growth of microorganism.

Hocheng et al [17] along with regular energy source, the bacteria cultural supernatant also used as additional tool to machine the Cu, Al, and Ni materials. The performance of the aforementioned tool is measured in terms of aspect ratio, remarks on the machined reveled that aspect ratio of the circle micro feature is very less than the straight line and square.

Istiyanto et al [18] brought the important observation in machining of micro feature such as lines, circle and rectangle shape on Cu, it is found that gear shape feature with detailed dimension of 25 micron could be possible to obtained.

Jadhav [19] used ferric sulphate produced biologically used as culture supernatant for machining copper metal. The effect culture supernatant plays a vital role along with process parameter to control the metal removal rate and surface roughness. The process parameter includes concentration of $FeSo_4$, shaking speed, quantity of cultural supernatant and machining time have shown pronounced effect on surface roughness and metal removal rate.

Mohammadreza Eskandarian et al [20] studies a enzymatic biomachining of copper was investigated. Material removal by chemical, microbial and enzymatic methods of 10h were 17.265g, 1.501g and 0.1005g, due to this results enzymatic machining has low rate and has good controllability and machining can be done effectively and the result show that the MRR is linear with time by adjusting reaction time. The rate of MRR was studied effectively by the enzymatic machining process.

Diaz - Tena et al [21] remove copper from OFC workpieces was investigated and it is categorized in to B1,B2,B3 and B4 (Cu concentration) of initial weight 2.071, 2.001, 1.925 and 1.849 and of final weight 2.001, 1.925, 1.849 and 1.777 was found and the MRR occurs high in the B3 sample of 18.7 mg/h cm^2 and the parameters of incubation, biomachining and regeneration phases are considered.

Imran Muhammad et al [22] enhances the optimum process parameters for maximum MRR by Taguchi methodology. From the investigation process the high MRR is achieved in the shaking rate of 160rpm, 45C temperature, 2.5pH and 16% Ferric concentration as a optimum process parameters.

Diaz - Tena et al [23] studied the material removal for OFC samples, From that the MRR was decreased with the effect of process parameters shows the increase in the concentration of trivalent ion and process temperature favours the loss of material under shaking conditions and the values of removal rate are five times higher in the first hour of test.

Abhishek Singh et al [24] investigates a biomachining on copper in the effect of various parameters were considered. From the experimental results of pH 2.5, shaking speed 180rpm, temperature 35C and machining time 32.71 hours shows the

material removal rate is maximum and also by the results of hardness test the hardness of workpiece doesn’t affected by the metallurgical properties of workpiece material.

3. RESEARCH GAP

- Machining of SMA (NiTi) with conventional machining process are available with certain extent.
- Also, some of the non-traditional machining process also attempted to obtain the desired shape and size.
- But all these process are having issues in terms of cutting tool wear or poor surface finish.
- Based on the literature survey, there is gap on understanding and identifying the suitable microorganism for machining the hard materials like SMA.
- The identified microorganism also requires suitable studies to identify the required conditions for performing the machining operation to get better surface finish.
- The proper modelling is also requiring to identify the proper process parameter for better material removal rate and surface finish.

4. OBJECTIVE

The following objectives were derived from the research gap.

- Identification of suitable parameter to fabricate Nickel, Titanium and Nitinol by Powder Metallurgy route.
- Identification of suitable condition to grow microorganisms' growth culture using various composition of salts.
- Biomachining on NiTi alloy will be done using two different types of microorganisms namely Acidithiobacillus Ferrooxidans and Acidithiobacillus Thiooxidans Microorganisms.
- The effect of machining of parameter such as pH value, concentration of ferric content, shaking time, and temperature are considered.
- To study the Material Removal Rate and Surface Roughness in biomachining.

5. METHODOLOGY

1

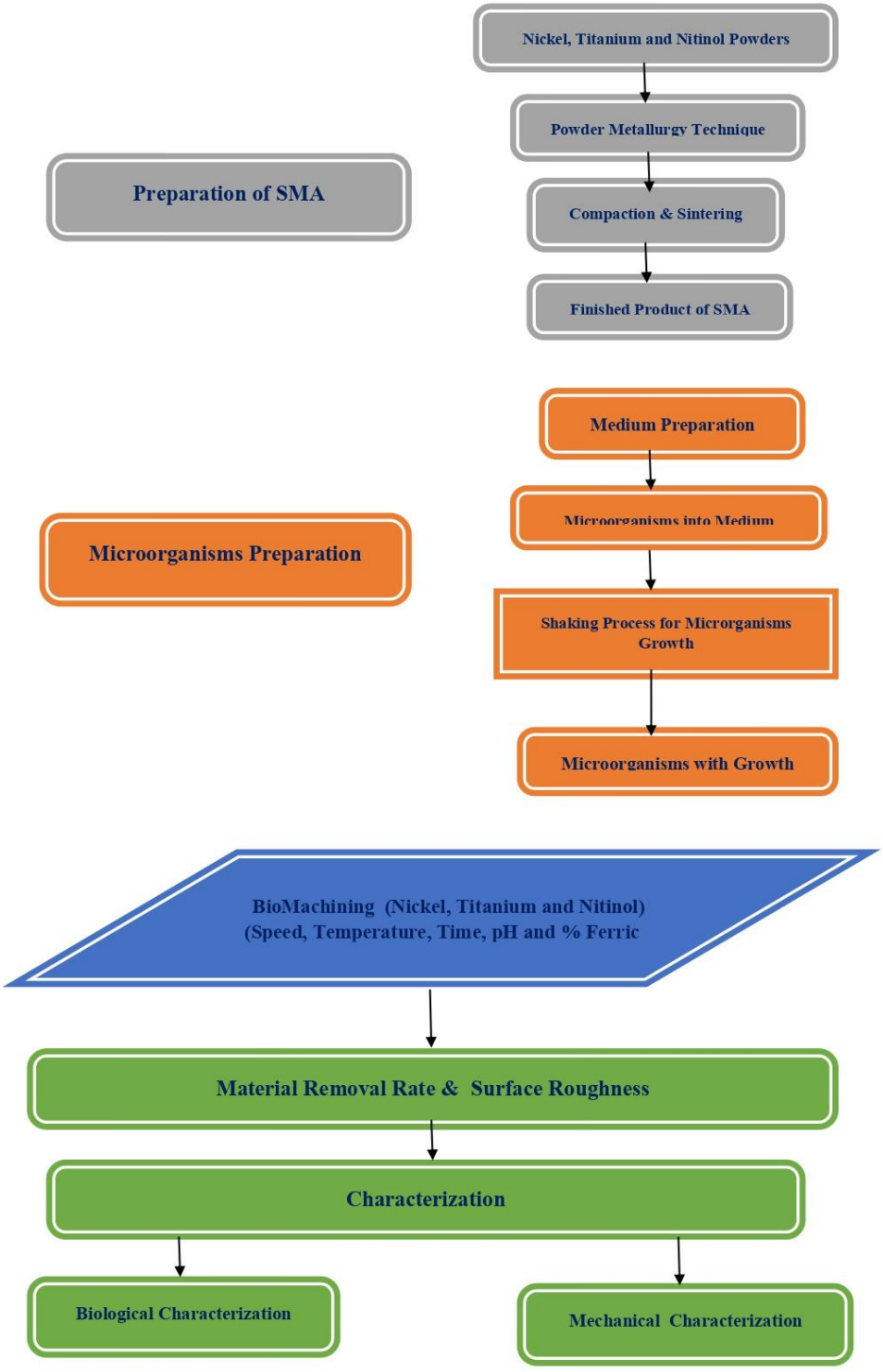


Figure.1. Methodology

6. Preparation of SMA, Microorganisms and BioMachining Process

Figure.1. shows the methodology adopted for the processing of Machining process and its characterization by step by step procedures. Nickel, titanium and nitinol specimens required for performing the bio-machining process based on the process parameters, are fabricated through powder metallurgy process. The required quantity of nickel, titanium, and nitinol powders were purchased from Parshwamani metals (Mumbai, India) with 99.2 %purity. The most suitable specimen size for bio-machining is selected and fixed as 10 mm diameter and 5 mm thickness. To fabricate the expected size, the required quantity of powder particle is measured and filled in EN32 oil hardened cylindrical steel die. The compaction pressure range is selected based on suitable compaction pressure. After the compaction process, the green specimen is removed and sintered in sintering furnace with respect to its melting point in the tubular furnace. After sintering process, the specimen is removed and cooled.

A pure culture of Acidithiobacillus Ferrooxidans, Thioxodans and Thiobacillus Novellus microorganisms are used for this bio-machining process. This culture was originally isolated from the medium of 250 ml which contains various composition of basal salts for the growth of microorganisms to machine SMA. Then the medium is to be sterilized by placing the 250ml conical flask in autoclave at 121 ℃ for 15 min, the sterilization is processed. After sterilization, inoculation of the culture was done in this medium by pouring of 1ml of Acidithiobacillus Ferrooxidans microorganisms by using micropipette. This culture was incubated at 30 ℃ under the stationary conditions for 24 h or kept in shaker at 140 rpm for 1-2 days until the solution becomes apparent as light yellow and semitransparent on the surface of media which will be identified as growth of Acidithiobacillus Ferrooxidans. All of these operations are conducted under the laminar chamber of the bacterial hood to avoid contamination.

Another process of Biomachining is carried out by with microorganisms and without microorganisms (i.e. various composition of basal salts) to determine Material Removal Rate and Surface Roughness.

The surface of the work samples was polished with different grits of silicon carbide (SiC) paper. Before biomachining, the work samples were cleaned using ethanol then dried and weighed. The culture was taken and the workpiece is placed in

Acidithiobacillus Ferrooxidans and Thiooxidans culture and this done in a laminar chamber, a closed chamber to avoid contamination.

All the prepared conical flask is sealed with cotton and placed in the shaker by setting different parameters of shaking speed, temperature, pH and presence of ferrous sulphate. Every 24 h the samples were taken out from the flask and dried in room temperature. The weight of the sample after machining was measured and reported. The weight of the sample before and after the experiment was measured using analytical digital weighing balance with an accuracy of 0.0001g. The following mathematical expression is used to find the MRR.

$$\text{MRR} = \left(\text{Initial wight of the work sample } (m_i) - \text{ Final weight of the sample } (m_f)\right)$$

7. RESULT AND DISCUSSION

7.1 Biological Characterization - Microorganisms

Cell viablity plays a key role in the bio machining process. The high bacterial cell concentrations would provide high metabolic activity which may supports indirect metal removal mechanism depends upon the materials, and the amount of cell concentration to oxidize the metal may varies. Consequently, fixed cell concentration in the culture is recommended to avoid a decreasing SMRR and to ensure optimal process parameter. The optical density values of microorganisms are measured using Haemocytometer and the cells are viewed through the microscope shown in Figure.7.1 and 7.2. The viable cell count method is used to determine the cell density of the microbial culture, and ISO20391-1 is used for measuring for cell count.

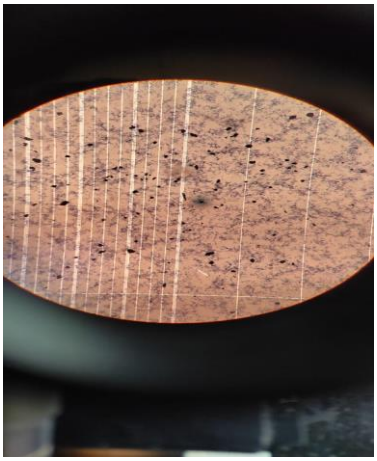


Figure. 7.1 Microscopic view of Cells

Figure. 7.2 Haemocytometer Slide

7.2 Material Removal Rate & Surface Roughness

By using these different range of parameters, higher and better material removal rate is identified and reported using Shimandzu ATY 224 analytical electronic weighing scale with a precision of 0.0001gm. The Zeiss TSK - Surfcom touch 50A contact type roughness tester is used to measure the Surface Roughness (Ra) value with the evaluation length of 5 mm, three trials are performed and the average value is reported.

MRR and R_a for Nickel, Titanium are taken, NiTi Shape Memory Alloy is used for the characterization and optimization process to undergo SMA studies to identify optimal parameter. The experiment is designed based on the Design of Experiment (DoE) three different bio-machining parameters are taken as input parameters: Shaking Speed, pH, and Temperature.

7.3 Optimization of Biomachining parameters Studies

The Taguchi approach is employed to perform the bio-machining studies. As shown in Table 7.1, three inputs with three levels requires 27 experiments to understand the effect of bio-machining process parameters. The experiments are planned as per the recommendations. The experiment is repeated thrice, the average value of MRR and R_a is taken for analysis.

To find the better operating parameter GWA algorithm is also proposed. The Grey Wolf Algorithm (GWA) optimization method is used to determine the importance of process parameters in achieving a greater SMRR and a better R_a. The reason for choosing GWA is that traditional swarm-based intelligence techniques fail to keep track of the process over time. In addition, GWA is unique because it has complete control over the entire area due to the grey wolves' leadership and hunting abilities. The following part covers the grey wolf hunting process, which is associated with constructing a mathematical model for hunting behaviour. The steps in hunting are as follows: it begins with pursuing, rushing, and approaching the prey. Then, it begins to pursue, encircle, and torment the victim once it is closed the prey. Finally, it pounces on its target. Each hunting behaviour is mathematically simulated by assuming that alpha grey wolves play the central role; as a result, alpha is a good

solution for the specific problem, followed by beta and delta. As a result, the optimum solution is determined by the alpha position, while the second and third choices are determined by the beta and delta positions. Omega's position is the least important in determining the solution. Therefore, it is feasible to decide on the wolves' hierarchy position throughout the encircling phase.

Factors	Level (1)	Level (2)	Level (3)
Shaking speed in rpm	80	120	160
Temperature in ^{o}C	25	30	35
pH	2.5	3.5	4.5

Table 7.1.Experimental Design.

7.4 Powders Characterization

The tap and apparent density are first measured according to ASTM B527-14, After that, the particle distribution of NiTi powder is determined using the Shimadzu SALD – 2300 laser diffraction technique as shown in Figure.7.3.

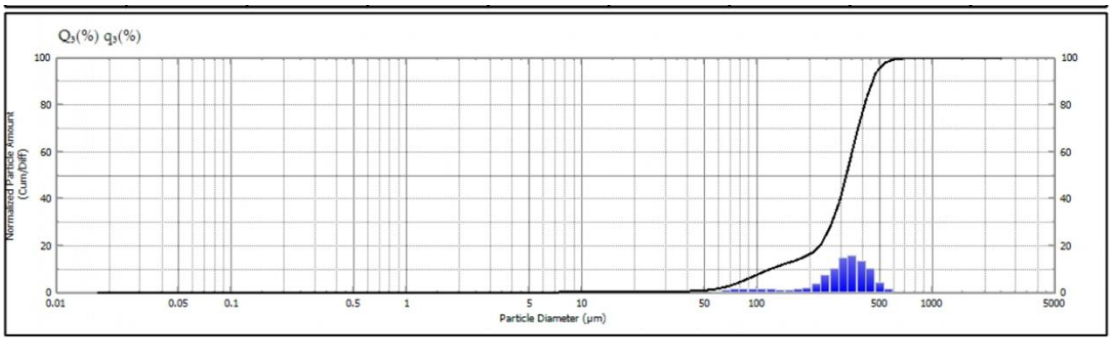
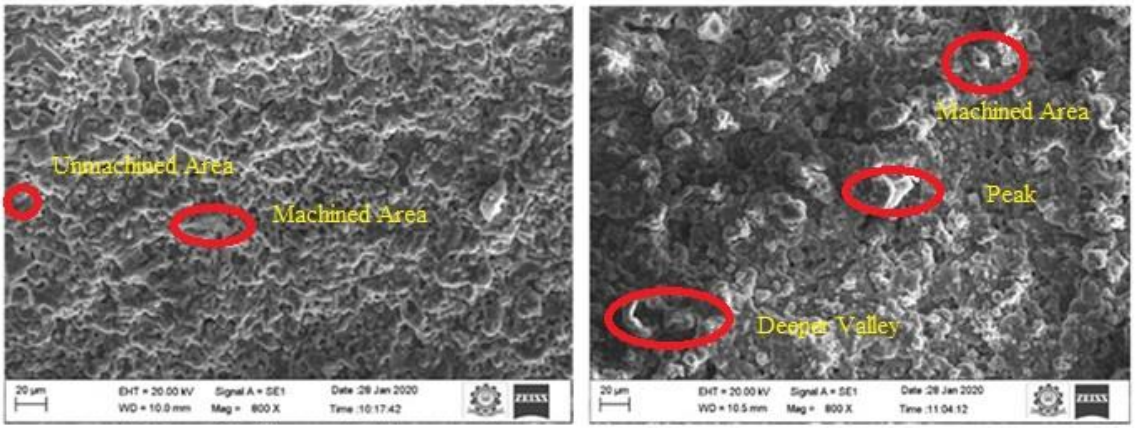


Figure. 7.3. Particle size of the powder

7.5 Machined Surface Characteristics

It is noted from the SEM image that the particles are uniformly distributed, compressed, and sintered and the surface of the specimen is machined with the microorganisms. Material Removal Rate starts from weakest section (ie) grain boundaries as a initial stage throughout the surface. It is compared with different temperatures, pH values and percentage of ferric content by keeping the shaking speed as constant. Shaking speed is one the essential parameter for obtaining fine MRR which shows the supply of nutrients spread throughout the medium aids in direct metal removal mechanism.

Figure.7.4 (a) and Figure.7.4(b) shows the machined and Unmachined area with machining of shape memory alloys with Acidithiobacillus Ferrooxidans and Thiooxidans microorganism, which shows the material removal and changes in surface roughness.

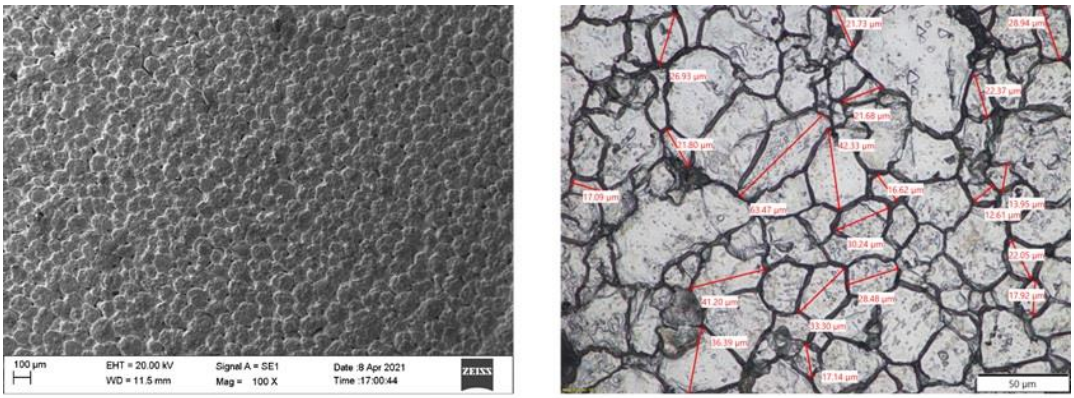


(a) (b)

Figure. 7.4 Machined surface of the Shape Memory Alloys

a) Acidithiobacillus Ferrooxidans b) Acidithiobacillus Thiooxidans

ASTM E 112 is used to measure the grain boundary after polishing and etching. The morphological aspect of the NiTi powder is studied using an SEM to understand the powder's morphology. The microstructure are taken for the NiTi SMA using Optical Microscope to for measuring grain size and grain boundaries.Figure.7.5(a) and Figure.7.5(b) shows the microstructure and grain boundaries of the fabricated specimen.



a) b)

Fig. 7.5 (a)SEM image of fabricated specimen, 7.5 (b) Grain size of the fabricated specimen

The provided research allows to show usefulness of bio-machining process for nickel, titanium and nitinol with Acidithiobacillus Ferrooxidans, Thiooxidans and Thiobacillus Novellus microorganisms. The obtained results confirm that Material Removal Rate and Surface Roughness is a performance indicator to check the bio-machining performance for the future scope compared with other machining process by considering various parameters like shaking speed, temperature, pH and percentage of ferric content.

Nickel, titanium, and nitinol materials are suitable for medical applications in which they are fabricated using the powder metallurgy technique and machined by biomachining process to finely finish their microstructure with its optimal compaction pressure and sintering temperature, no heat generation and with fine dimensional accuracy.

Material removal rate and Surface roughness are investigated to obtain better material removal rate and surface roughness by considering various parameters like Shaking speed, temperature, pH and percentage of ferric content.

These biomachining process proved that Material Removal rate and Surface roughness can be improved by optimizing the process parameters should be considered for implant materials in biomedical applications and this biomachining process can be replaced by other conventional and unconventional machining process.

8. ORGANISATION OF THESIS

The organization of the thesis is as below:

Chapter 1: This chapter introduces the Shape memory alloys and micromachining concept for the biomedical applications with lower energy resources, fine dimensional tolerances and no heat affected zone. Shape memory alloys (Nickel, Titanium and Nitinol) are used for making product for the experimental work. Biomachining is employed to achieve the above parameters which is a challenge for the conventional and Unconventional machining process. For Biomachining process, four parameters like shaking speed, time, pH value and temperature are considered.

Chapter 2: In this chapter an elaborated literature is reviewed based on the recent publications focused on biomachining and other conventional and Unconventional machining process. Material Characterization are also reviewed, what are the tests are to carried for biomachining with respect to the applications. As well as, the research gap of previous kinds of literatures and the main objective of our present research work is reported with the proper methodological manners.

Chapter 3: In this chapter covered the detailed information regarding preparation of Shape Memory Alloys – Nickel, Titanium and Nitinol by using Powder Metallurgy Technique. Number of samples are planned and fabricated using powder metallurgy process with appropriate compaction pressure and Sintering temperature for machining with Acidithiobacillus Ferrooxidans, Thiooxidans and Thiobacillus Novellus microorganisms and polishing process before machining are also discussed.

Chapter 4: This chapter briefly discussed preparation of microorganisms by inoculating the chemicals with Acidithiobacillus Ferrooxidans, Thiooxidans and Thiobacillus Novellus microorganisms. Acidithiobacillus Ferrooxidans, Thiooxidans, Thiobacillus Novellus was purchased from SOM Phytopharma Limited (Hyderabad, India). The culture is in the liquid form and is packed in plastic cans. This culture was originally isolated from the medium contains various composition of basal salts and it is sterilized. After inoculation, microorganisms are poured in the medium and incubated at 30℃ for 24h or kept in a shaker for the growth. All these process are done in the laminar chamber to avoid contamination are discussed.

Chapter 5: This chapter discussed the biological characterization of prepared microorganisms by conduction cell viability tests which shows the number of cells present in the medium by using haemocytometer and the growth of microorganisms are derived by considering the optical density values.

Chapter 6: This chapter briefly discussed the biomachining process by considering the various parameters like shaking speed, time, pH value, temperature

and percentage of ferric content. Biomachining process is carried out in a shaker by considering the above said parameters to find the Material Removal Rate and Surface Roughness and also the suitable shape memory alloy is to identified by considering better Material Removal Rate and Surface Roughness. Material Removal Rate is measured using weighing balance and surface roughness is measured using Zeiss TSK - Surfcom touch 50A contact type roughness tester.

Chapter 7: This chapter explained the characterization of machined samples like Nickel, Titanium and Nitinol with Acidithiobacillus Ferrooxidans and Thiooxidans microorganism. Machined area, Unmachined area, grain boundaries and powder propagation are identified by taking its microstructure in Scanning Electron Microscope and Optical Microscope.

Chapter 8: Based on the result and discussions of the experimental investigation of of Shape Memory Alloys with Acidithiobacillus Ferrooxidans and Thiooxidans microorganisms by performing biomachining process, Material Removal Rate and Surface Roughness are measured and it is reported in detail in the conclusion.

9. REFERENCES

1. Saeed Daneshmand, Reza Hessami, Habib Esfandiar, Investigation of Wire Electro Discharge Machining of Nickel-Titanium Shape Memory Alloys on Surface Roughness and MRR, *Life Science Journal*, **9** (4) (2012).
2. A.P. Markopoulos, I.S. Pressas and D.E. Manolakos, A Review On The Machining Of Nickel-Titanium Shape Memory Alloys, *Advanced Material Science* , **42** (2015), 28-35.
3. Yuebin Guo , Andreas Klink, Chenhao Fu, John Sny , Machinability and surface integrity of Nitinol shape memory alloy, *CIRP Annals - Manufacturing Technology*,(2013).
4. Ramesh Kuppuswamy, Akinori Yui, High-speed micromachining characteristics for the NiTi shape memory alloys, *International Journal Advanced Manufacturing Technology*, Springer (2015).
5. C. Velmurugan, V. Senthilkumar, S. Dinesh & D. Arulkirubakaran, Machining of NiTi-shape memory alloys-A review, *Machining Science and Technology*, **22** (3) *(2018)*, 355 – 401.
6. M.R. Hassan, M. Mehrpouya and S. Dawood, Review of the Machining Difficulties of Nickel-Titanium Based Shape Memory Alloys, *Applied Mechanics and Materials,* **564** (2014) , 533-537.
7. K. Weinert, V. Petzoldt, Machining of NiTi based shape memory alloys, *Materials Science and Engineering* , **378** (2004) , 180–184.
8. Himanshu Bisaria & Pragya Shandilya, Processing of curved profiles on Ni-rich nickel–titanium shape memory alloy by WEDM, *Materials and Manufacturing Processes*.(2019).
9. M. Manjaiah, S. Narendranath, S. Basavarajappa , Review on Non-Conventional Machining of Shape Memory Alloys, *Transactions of Nonferrous Metals. Society of China,* **24** (2014), 12 – 21.
10. W. Theisen, A. Schuermann , Electro discharge machining of nickel–titanium shape memory alloys, *Materials Science and Engineering,* **A 378** , 200–204.
11. Yoshiyuki Uno, Toshiaki Kaneeda , Seichi Yokomizo, Fundamental Study on Biomachining *Japan Society of Mechanical Engineers,* **39** (4), 1996.

12. Pallvi Verma, Amanpreet Kaur Sodhi and Neeraj Bhanot, Application of Aspergillus Niger for Biomachining of Aluminium Alloy 4004, *Springer Nature Singapore Pte Ltd,* (2019), 127-132.
13. Miyano Y, Kamiya O, Chouanine L, Fundamental studies on biomachining of carbon steel by iron oxidizing bacteria, *Trans JWRI*, **32** (2003), 239–242.
14. Daniel Johnson, Roscoe Warner, Albert Shih, Surface Roughness and Material Removal Rate in Machining using Microorganisms, *Journal of Manufacturing Science and Engineering,* **129** (1) (2007), 223-227.
15. J.H. Chang, H. Hocheng, H.Y. Chang, A. Shih, Metal removal rate of Thiobacillus thiooxidans without pre-secreted metabolite, *Journal of materials processing technology,* **20** (1) (2008), 560–564.
16. Jos Istiyanto, Tae Jo Ko, Il-Chae Yoon, A Study on Copper Micromachining Using Microorganisms, *International Journal Of Precision Engineering And Manufacturing,* **11** (5) (2010), 659-664.
17. Hong Hocheng , Jei-heui Chang, Umesh U. Jadhav, Micromachining of various metals by using Acidithiobacillus ferrooxidans 13820 culture supernatant experiments, *Journal of Cleaner Production,* **20** (2012), 180-185.
18. Jos Istiyanto, Agung-Shamsuddin Saragih, Tae Jo Ko, Metal based micro-feature fabrication using biomachining process, *Microelectronic Engineering* , **98** (2012), 561–565.
19. Umesh Jadhav, Hong Hocheng, Extraction of silver from spent silver oxidezinc button cells by using Acidithiobacillus ferrooxidans culture supernatant, *Journal of Cleaner Production,* **44** (2013), 39-44.
20. Mohammadreza Eskandarian , Afzal Karimi , Mohammadreza Shabgard, Studies on enzymatic biomachining of copper by glucose oxidase, *Journal of the Taiwan Institute of Chemical Engineers,* **44** (2013), 331-335.
21. E. Díaz-Tena , A. Rodríguez-Ezquerro , L.N. López de Lacalle Marcaide , L. Gurtubay Bustinduy , A. Elías Sáenz, A sustainable process for material removal on pure copper by use of extremophile bacteria, *Journal of Cleaner Production, (2014),* 1-9.
22. Imran Muhammad, Sahar M. Sana Ullah, Do Sup Han, Tae Jo Ko, Selection of Optimum Process Parameters of Biomachining for Maximum Metal Removal Rate, *International Journal Of Precision Engineering And Manufacturing-Green Technology,* **2** (4) (2015), 307-313.

23. E. Díaz-Tenaa,∗, G. Gallasteguib, M. Hipperdingerc, E.R. Donatic, M. Ramírezd,A. Rodrígueze, L.N. López de Lacallee, A. Elías, New advances in copper biomachining by iron-oxidizing bacteria, *Corrosion Science,* **112** (2016), 385–392.
24. Abhishek Singh, N. Arul Manikandan, M. Ravi Sankar, K. Pakshirajan, L. Roy, Experimental Investigations and Surface Morphology of Bio-Micromachining on Copper, Materials Today: *Proceedings,* **5** (2018), 4225–4234.

10. LIST OF PUBLICATIONS

1. M.Pradeep, Rajesh Shanmugavel, M.Uthayakumar, Lakshmanan Muthulakshmi, M.Adam Khan, Senthil Muthukumar Thiagamani, Sanjay Mavinkere Rangappa, Suchart Siengchin(2022), Experimental studies on biomachining process using novel Thiobacillus novellus microorganism – a comparative study", Biomass Conversion and Biorefinery – Springer Nature.

2. M. Pradeep, S. Rajesh M. Uthayakumar, P. Sivaranjana, S. Syath Abuthakeer, M. Ravichandran, Senthil MuthuKumar Thiagamani, Sanjay Mavinkere Rangappa, Suchart Siengchin(2022), "Investigations on the combined effects of Thiobacillus Novellus microorganism and process parameters on the bio-machining of NiTi." Biomass Conversion and Biorefinery – Springer Nature.

3. Mani Pradeep, Shangumavel Rajesh, Marimuthu Uthayakumar, Chandrasekar Mathalai Sundaram, Kinga Korniejenko, Krzysztof Miernik, Mohd Shukry Abdul Majid,(2023) **"**Experimental Investigation on Bio-Machining of Nickel, Titanium and Nitinol (Shape Memory Alloys) Using Acidithiobacillus ferrooxidans Microorganisms", Journal of Composites Science.

4. M.Pradeep, Rajesh Shanmugavel, M.Uthayakumar, "Biomachining of Acidithiobacillus Ferrooxidans Microrganisms with Titanium by using Powder Metallurgy", A book chapter in Advances in Sustainable Manufacturing – Universiti Putra Malaysia.